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Original Article

Rats treated with *Hypericum perforatum* during pregnancy generate offspring with behavioral changes in adulthood

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ABSTRACT

Drugs used in the treatment of depression can cross the placenta giving rise to questions regarding the effects these drugs exert on the fetus. *Hypericum perforatum* L., Hypericaceae, is a natural product used to treat depression. However, information about its toxicity and the occurrence of alterations in the central nervous system development of the offspring is scarce. This work assessed the behavior of adult male rats born from mothers treated with *Hypericum* extract during gestation and analyzed the fluorescence of the extract in different organs of mothers and fetuses. Male pups were divided into three treated groups, corresponding to the administration of the *Hypericum* extract to mothers at the dose levels of 36 mg/kg, 72 mg/kg and 144 mg/kg, and one control group in which the mothers received distilled water. At 90 days of age, the offspring underwent the following tests: rotarod, pentobarbital-induced sleep time, elevated plus maze, hole-board and forced swimming test. The observed fluorescence indicated the presence of the extract in all tissues analyzed. The obtained results suggest lasting changes in the performances displayed in the CNS, depression and anxiety tests, indicating that the use of *Hypericum* during gestation could interfere with the behavioral development of the offspring reducing anxiety and depression when they become adults. We suggest that these alterations are associated with the reprogramming of the brain regions related to changes in emotional reactivity.

Introduction

Depression is the most prevalent mental disorder in pregnant women, whose incidence is progressively increasing over the years (Bennett et al., 2004; Pereira and Lovisi, 2008). The neuroendocrine changes that take place during gestation are considered risk factors for the appearance of depressive disorders, and contrary to popular belief, the gestational period does not protect the women's mental health (Campagne, 2004; Camacho et al., 2006). The gestational depressive disorder alters the maternal environment and produces severe damages to the health of the mother and child, such as decreased quality of life, puerperal depression and anxiety setting, obstetric complications, gestational hyperemesis, premature birth, low fetus weight, increased hospitalization in the neonatal intensive care unit and interference in the newborn's development (Oberlander et al., 2006; Field et al., 2008). All these factors emphasize the importance of the treatment of gestational depression as a prenatal care measure.

The tricyclic antidepressants, the monoamine oxidase inhibitors (MAOI) and the selective serotonin reuptake inhibitors (SSRI) are different classes of synthetic medications used in the treatment of depression during pregnancy known to improve 60 to 70% of the symptoms in 30 days. However, these drugs also produce undesirable side effects, including sexual dysfunction, hallucinations, delirium, risk of maniac episodes, gastrointestinal disorders, anticholinergic effects, sedation and intoxication (Souza, 1999). In addition, preclinical studies have shown that they increase the risks of teratogenicity, can cross the placenta and can be found in the amniotic fluid (Hostetter et al., 2000).

The tolerance developed by patients after chronic exposure to some antidepressants and anxiolytics is mainly characterized by the reduction in the number of serotonin (5-HT), noradrenalin (NOR) and gamma-aminobutyric acid (GABA) receptors. Therefore,

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their use by pregnant women could affect fetal neurodevelopment, since the formation of the central nervous system (CNS) continues for an extended period postnatally (Murrin et al., 2007).

Hypericum perforatum L., Hypericaceae, is popularly known as Hyperic or St John's wort and is a well-known antidepressant (Schmidt and Butterweck, 2015) that also shows antinociceptive and anticonvulsant effects (Galeotti et al., 2014) and others (Linde, 2009). *Hypericum perforatum* (Hp) can be found distributed across several continents: Europe (Germany, Russia, Poland), West Asia, North Africa, North America, South America and Australia. It is considered efficacious and safe when compared to synthetic medications (Linde et al., 2008, 2009; Howland, 2010; Nahas and Sheikh, 2011), since its use does not cause the typical side effects of psychoactive substances such as sedation, memory loss, addiction and (Brattstrom, 2009) intolerance. No teratogenic effects have been reported when taken by pregnant women, however its use should be done with caution as the action of *H. perforatum* on the maternal and fetal organism as well as the future consequences on the behavior of the offspring have not been fully elucidated (Tschudin and Lapaire, 2005; Dugoua et al., 2006).

Hypericum perforatum extract is a phytocomplex composed of various substances, including hypericin, pseudohypericin, hyperforin, flavonoids (quercetin, hyperoside and rutin), tannins and procyanidins. Various concentrations have been identified in the commercial products of the extract (Bergonzi et al., 2001), however what defines the standard extract is the concentration of hypericin which should be 0.3% (Muller et al., 1997). The antidepressive effect of Hp extract can be attributed to the isolated or synergistic action of the substances present in it (Reichling et al., 2003), but several reports indicate that hypericin, pseudohypericin, the flavonoids and hyperforin may be the main components responsible for this effect (Xu et al., 2005; Borrelli and Izzo, 2009). In particular, hyperforin is known to inhibit the reuptake of 5-HT, NOR, dopamine and glutamate and to interfere with the neuronal efflux and influx of electrolytes (Bouron and Lorrain, 2014).

Owing to the knowledge that Hp extract presents fluorescent constituents, such as hypericin and pseudohypericin (Draves and Walker, 2000), this work employed the *in vivo* imaging system to determine the biodistribution of the extract on the placenta and on the maternal and fetal organisms. In addition, this work also evaluated the behavior of adult male rats born from mothers treated with Hp extract during gestation.

Material and methods

Plant material and extract

The standard dry extract of *Hypericum perforatum* L., Hypericaceae, containing 0.3% of hypericin, was imported from China and identified by number of deposit 20100913. The extract was then prepared by Mbpharma Manipulações Ltda (Matias Barbosa, Brazil), lot 10124778E and stored at temperatures between 15 °C e 25 °C in a dark glass vial.

Animals and treatment

One-day pregnant Wistar rats (*Rattus norvegicus*) (90 days old), were obtained from the vivarium of the Center for Reproductive Biology of the Federal University of Juiz de Fora and housed in polypropylene cages (40 × 30 × 16 cm), containing five animals each. They were kept under standard laboratory conditions, with controlled temperature of 23 ± 2 °C, and a 12 h light/dark photoperiod, with the light period beginning at 6 am. The dams had free access to water and were fed on rat chow pellets (Nuvilab® -Paraná, Brazil).

The dams were randomly distributed into four groups of ten pregnant rats: three treated groups and a control one, and underwent treatment during the gestational period once daily. The treated groups received orally (gavage) the aqueous extract of Hp at doses of 36 mg/kg (T1), 72 mg/kg (T2), and 144 mg/kg (T3) of body weight. The control group (C) received distilled water during the same period. Treatment of mothers was discontinued after delivery. The choice of doses was based on the information available in the literature, taking into account that the action of Hp in the central nervous system (CNS) of rats was previously observed at doses higher than 30 mg/kg (Crupi et al., 2011).

One day before giving birth, the rats were placed in individual cages. After weaning, the male offspring were divided into four groups: T1, T2, T3 and C, corresponding to the treatments received by their respective mothers. At 90 days of age all male pups were weighed and ten animals from each group, comprising random selection of one descendent from each mother, were subjected to the rotarod test and to the pentobarbital-induced sleep time test seven days later. Ten other males from each group were subjected to the elevated plus maze test and a week later to the forced swim test. In a similar way, ten other animals were evaluated in the hole-board test. The female pups were studied separately and the results will be presented elsewhere.

The project was approved by the Ethical Committee in Animal Experimentation of the Federal University of Juiz de Fora (protocol number 003/2011).

Motor performance test (rotarod test)

Motor performance was measured as time spent walking on a rotating rod (7 rpm) in just one trial, *i.e.*, after falling from the bar the animal was returned to the cage. To this effect, the animals were submitted to a preselection 24 h before testing and only those remaining on the revolving bar of the rotarod for at least 60 s in one out of three trials were selected (Kannan et al., 2013). The equipment used was a rotarod Panlab s.l. mod. LE 8200 (Barcelona, Spain).

Pentobarbital-induced sleep time

The animals were injected *i.p.* with sodium pentobarbital (Syntec, Hypnol^{R}; 40 mg/kg, Juiz de Fora, Brazil) and the latency (in seconds) – interval between the pentobarbital administration and the beginning of the sleeping time – and the time (in seconds) between loss and recovery of the righting reflex were recorded (Wambebe, 1985)

Elevated plus maze test

The elevated plus maze apparatus (Novalab; Ribeirão Preto, Brazil) consisted of four arms (50 cm) elevated 39 cm above the floor. Each arm was positioned at 90° relative to the adjacent arms and all arms were connected through a central area (5 × 5 cm), forming a plus sign. Each rat was placed at the center of the maze facing one of the open arms. The time spent (in seconds) in the open arms was recorded for 3 min. Entry into an arm was defined as the animal having crossed with all four paws the dividing line between the central area and the arm (Herrera-Ruiz et al., 2008; Grundmann et al., 2009; Han et al., 2009). After each trial, the plus maze was carefully cleaned with 10% ethanol solution.

Hole-board test

The hole-board apparatus (Panlab s.l. mod. LE 8811, Barcelona, Spain) consisted of a transparent acrylic box with sixteen equidistant holes on the surface with photoelectric cells on the side plates.

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